Effect of phloretin on growth performance, serum biochemical parameters and antioxidant profile in heat-stressed broilers

Hong Hu,[*,†,1] Xi Bai,[†,1] Kexing Xu,[†] Cheng Zhang,[‡] and Liang Chen[*,2]

[*]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China;* [†]*College of Animal Science, Anhui Science and Technology University, Chuzhou 233100, China; and* [‡]*College of Animal Science and Technology, Anhui Agricultural University, Hefei, Anhui, China*

ABSTRACT The objective of this work was to evaluate the effect of phloretin on growth performance, serum biochemical parameters, antioxidant profile, glutathione (**GSH**)-related enzymes, nuclear factor erythroid 2 −related 2 (**Nrf2**) and heat shock protein 70 (**HSP70**) in heat-stressed broilers. A total of 240, 22-day-old Arbor Acres broilers were divided into 4 groups. The control group was housed at 23.0 ± 0.61°C and fed with basal diet, while the 3 heat-stressed groups (A, B, and C groups) were housed at 30.5 ± 0.69°C and fed with basal diet containing 0, 100, and 200 mg/kg phloretin, respectively. Serum was taken form 42-day-old broilers. Results showed that heat stress decreased ($P < 0.05$) the final body weight (**FBW**), body weight gain (**BWG**), feed intake (**FI**), serum total protein (**TP**), triglyceride (**TG**), triiodothyronine (T3), thyroxine (**T4**), GSH, catalase (**CAT**), and total antioxidant capacity (**T-AOC**) levels, but increased ($P < 0.05$) the feed-to-gain ratio (**FGR**) and serum malondialdehyde (**MDA**) levels in broilers compared with that in the control group. Among the heat-stressed groups, supplementary 200 mg/kg phloretin increased ($P < 0.05$) the FBW, BWG, FI, serum TP, TG, T4, GSH, CAT, and T-AOC levels, and decreased ($P < 0.05$) the FGR and serum MDA in broilers. There were significant decreases ($P < 0.05$) in the glutathione peroxidase (**GSH-Px**), γ-glutamylcysteine synthetase (**γ-GCS**), and Nrf2, but significant increases ($P < 0.05$) in the HSP70 of the broiler serum after heat stress treatment. Among the heat-stressed groups, supplementary 200 mg/kg phloretin increased ($P < 0.05$) the GSH-Px, γ-GCS, and Nrf2 levels, but decreased ($P < 0.05$) the serum HSP70 level in the heat-stressed broilers. Under high temperature condition, FBW, BWG, FI, FGR, serum TP, TG, T4, MDA, GSH, CAT, T-AOC, GSH-Px, γ-GCS, Nrf2 and HSP70 were linearly affected by inclusion of phloretin. These results indicated that phloretin may improve growth performance, serum parameters, and antioxidant profiles through regulated GSH-related enzymes, Nrf2 and HSP70 in heat-stressed broilers.

Key words: Phloretin, heat stress, broiler, growth performance, serum antioxidant profile

2021 Poultry Science 100:101217
https://doi.org/10.1016/j.psj.2021.101217

INTRODUCTION

High summer temperatures have become a universal problem in high-density and intensive poultry industry (Farag and Alagawany, 2018; Nawab et al., 2018). Owing to their fast growth rate, strong metabolism, and lack of sweat glands, broilers have a low tolerance to high temperature and humidity environments, which leads to a significant decrease in growth performance and increase in mortality (Nawab et al., 2018; Goo et al., 2019; Shakeri et al., 2019; Slawinska et al., 2020). Several studies have shown that high temperature will lead to oxidative stress in broilers (Akbarian et al., 2016; Ghanima et al., 2020). Therefore, antioxidants that relieve oxidative damage can improve thermal stress in broilers.

Phloretin, extracted from fruits (including apples, pears, and peaches), leaves, trees, and various vegetables, is a type of bioactive flavonoid (Wang et al., 2018; Shao et al., 2008). Phloretin has many biological activities, including antioxidant, hypoglycemic, protecting blood vessels, improving immunity, and antitumor functions (Chang et al., 2012; Barreca et al., 2014; Lin et al., 2014). In particular, it can neutralize the active free radicals in the cytoplasm and improve the overall ability of cells to resist oxidative stress (Zuo et al., 2011; Mendes et al., 2018). Recent research has shown that phloretin can enhance the antioxidant activity of the

Received August 20, 2020.
Accepted April 15, 2021.

[1]These authors contributed equally to the experimentations.

[2]Corresponding author: chenliang01@caas.cn

body by regulating cells in various pathways (Behzad et al., 2017). As a new, natural, and high-efficient antioxidant, phloretin has been approved for use in several fields such as medicine, cosmetics, and food processing (Behzad et al., 2017; Wang et al., 2020). However, only few studies have discussed the use of natural sources of phloretin as a new type of green antioxidant feed additive in broiler production.

Serum metabolites are important indicators of metabolism and changes in specific tissues and organs in broilers during heat stress (Ghazi et al., 2015). Few studies have investigated the capacity of phloretin to alleviate the oxidative state in broiler serum and related mechanisms caused by heat stress. The objective of the present study was to evaluate the effect of supplementary phloretin on growth performance, serum biochemical parameters, and antioxidant profiles in broilers reared in a hot environment (Chinese summer conditions). Furthermore, we aimed to evaluate the regulation of the glutathione (**GSH**)-related enzymes, nuclear factor erythroid 2−related 2 (**Nrf2**) and heat shock protein 70 (**HSP70**), to identify the possible mechanism by which dietary phloretin protects growth performance, serum parameters, and oxidative stress in broilers exposed to hot environments.

MATERIALS AND METHODS

Experimental Birds and Diet

All experiments were approved by the Animal Care and Use Committee of Anhui Science and Technology University. Broilers (Arbor Acres) were obtained from the farm of the Anhui Science and Technology University. A total of 240, 22-day-old broilers (half males and females) were divided into 4 groups (10 birds/replicate and 6 replicates/group). Broilers in the control group were housed in the thermoneutral condition (Table 1), and broilers in the heat-stressed groups (A, B, and C groups) were housed in the hot environment (Table 1) under summer conditions from June 14 to July 4, 2019. The thermoneutral condition was controlled by automatic temperature control system. The temperature and humidity of heat-stressed groups were recorded every 2 h (Table 1).

Broilers in the control and A groups were fed the same basal diet, and broilers in the B and C groups were fed the basal diet supplemented with 100 and 200 mg/kg phloretin (purity: ≥98%; Aladdin Reagent Co., Ltd., Shanghai, China). The basal diet (Table 2) was designed to meet the requirements specified by the NRC (1994). Birds had free access to water and diet, and enjoyed a 12-h light regimen in this experiment. The size of stereoscopic cage was 120 × 70 × 40 cm. The content of phloretin was measured by improved HPLC method based on Li et al., 2013 (Table 2).

Table 1. The treatment of broilers.

Groups	Temperature (°C)[1]	Humidity (%)[1]
Control group	23.0 ± 0.61	50.8 ± 5.41
Heat-stressed group	30.5 ± 0.69	89.7 ± 6.70

[1]The temperature and humidity were recorded every 2 h. The values of temperature and humidity were exhibited by mean ± standard deviation.

Table 2. Ingredients and nutrient analysis of the basal diets (as-fed basis).[1]

Ingredients (%)	22−42 d[1]
Corn	60.0
Soybean meal	31.2
Fish meal	2.0
Soybean oil	3.0
$CaHPO_4{\cdot}2H_2O$	1.5
Limestone	0.9
Salt	0.3
DL-Met	0.1
Premix[2]	1.0
Calculation of nutrients	
Metabolizable energy (MJ/kg)	12.56
Crude protein (%)	20.26
Calcium (%)	0.87
Total phosphorus (%)	0.41
Analyzed composition	
Crude protein (%)	20.10
Calcium (%)	0.91
Total phosphorus (%)	0.49
Phloretin (mg/kg)	4.36

[1]Broilers in the control and A groups were fed the basal diet, and broilers in the B and C groups were fed the basal diet supplemented with 100 and 200 mg/kg phloretin.

[2]Supplying per kilogram of diet: Fe: 60 mg, Cu: 5 mg, Zn: 50 mg, Se: 0.1 mg, I: 0.3 mg, vitamin A: 10, 000 IU, vitamin K: 4.0 mg, cholecalciferol: 2200 IU, vitamin E: 20 mg, vitamin B1: 2.0 mg, vitamin B2: 4.0 mg, vitamin B6: 4.0 mg, vitamin B12: 0.03 mg, niacin: 20 mg, pantothenic acid: 10 mg, folic acid: 1.2 mg, biotin 0.12 mg; choline 400 mg.

Sample Collection

On 42 d of age, 12 birds per group (2 birds from each replicate) were selected and slaughtered after overnight fasting in the present study. The birds were killed by exsanguination. The blood sample of broiler was collected. Individual serum sample was separated by centrifuged at 3500 rpm for 12 min under 4°C condition and then stored at −70°C for detecting serum biochemical parameters, oxidative state, GSH-related enzymes, Nrf2 and HSP70 levels.

Growth Performance Analysis

The initial body weight, final body weight (**FBW**), body weight gain (**BWG**), feed intake (**FI**), and feed-to-gain ratio (**FGR**) were measured and calculated as described by Hu et al. (2019). The body weight of the broilers was measured every week. The FI was measured by calculating the difference between the supplied and remaining feed every day. BWG was calculated by the difference between the weight of 22 and 42 day-old-broilers. FGR was expressed as FI: BWG.

Serum Biochemical Parameters Analysis

The total protein (**TP**), glucose, and triglyceride (**TG**) levels in the serum were determined using a Total

Protein Assay Kit (A045-4-2; with standard: BCA method), Glucose Assay Kit (F006-1-1), and Triglyceride Assay Kit (A110-2-1) which were purchased from Jiancheng Bioengineering Institute Co., Ltd. (Nanjing, China), respectively. The triiodothyronine (T3) and thyroxine (T4) levels in the serum were determined using Chicken T3 ELISA Kit (JYM0114Ch) and T4 ELISA Kit (JYM010Ch) which were purchased from Jiyinmei Biotechnology Co. Ltd. (Wuhan, China), respectively.

Serum Oxidation State Analysis

The serum malondialdehyde (**MDA**) was determined by Micro Malondialdehyde Assay Kit (BC0025; Solarbio Technology Co. Ltd, Beijing, China). The serum superoxide dismutase (**SOD**) was determined by Superoxide Dismutase Assay Kit (SOD-1-Y; Comin Biotechnology Co. Ltd, Suzhou, China). The serum GSH, catalase (**CAT**), and total antioxidant capacity (**T-AOC**) were determined by Glutathione Assay Kit (A006-2-1), Catalase Assay Kit (A007-1), and Total Antioxidant Capacity Assay Kit (A015-2-1) from Jiancheng Bioengineering Institute Co., Ltd (Nanjing, China).

GSH-Related Enzyme Analysis

The glutathione peroxidase (**GSH-Px**) and γ-glutamylcysteine synthetase (**γ-GCS**) were determined by Glutathione Peroxidase Assay Kit (Colorimetric method) and γ-Glutamylcysteine Synthetase Assay Kit (Jiancheng Bioengineering Institute Co., Ltd, Nanjing, China).

Nrf2 and HSP70 Analysis

The Nrf2 and HSP70 levels were determined by Chicken Nrf2 and HSP70 enzyme-linked immunosorbent assay kit (Jiancheng Bioengineering Institute Co., Ltd, Nanjing, China).

Statistical Analysis

The data in the present study were analyzed using SPSS 18.0 software (SPSS Inc., IL). The t test was performed to compare the control and A groups. A one-way analysis of variation and Tukey's test were used to determine the differences among the A, B, C groups. The linear and quadratic effects of phloretin were detected by orthogonal polynomials. Values are expressed as means ± SEM and $P < 0.05$ was regarded as significant.

RESULTS

Growth Performance

There were no significant differences in initial body weight among 4 groups (Table 3). Heat stress (A group) decreased ($P < 0.05$) the FBW, BWG and FI, but increased ($P < 0.05$) the FGR in broilers compared with that in the control group (Table 3). As shown in Table 3, broilers in the B group (heat stress + 100 mg/kg phloretin treatment) exhibited higher FBW and BWG, but lower FGR than those in the A group (heat stress treatment); broilers in the C group (heat stress + 200 mg/kg phloretin treatment) exhibited higher FBW, BWG and FI, but lower FGR than those in the A group (heat stress treatment). Under high temperature condition, FBW, BWG, FI, and FGR were linearly affected by inclusion of phloretin (Table 3).

Serum Biochemical Parameters

Heat stress (A group) decreased ($P < 0.05$) the serum TP, TG, T3, and T4 levels in broilers compared with that in the control group (Table 4). As shown in Table 4, broilers in the C group (heat stress + 200 mg/kg phloretin treatment) exhibited higher serum TP, TG, and T4 than those in the A group (heat stress treatment). Under high temperature condition, serum TP, TG, and T4 were linearly affected by inclusion of phloretin (Table 4).

Table 3. Effect of phloretin on growth performance in broilers (22−42 d) exposed to hot environment.

Item	Control group[1]	Heat-stressed groups[2]			P-Value	
		A	B	C	Linear[3]	Quadratic[3]
IBW (kg)	0.617 ± 0.004	0.613 ± 0.003	0.614 ± 0.002	0.611 ± 0.004	0.740	0.581
FBW (kg)	2.110 ± 0.052*	1.782 ± 0.017[a]	1.894 ± 0.034[b]	1.971 ± 0.031[b]	< 0.001	0.624
BWG (kg)	1.492 ± 0.054*	1.170 ± 0.018[a]	1.280 ± 0.035[b]	1.360 ± 0.031[b]	< 0.001	0.669
FI (kg)	3.061 ± 0.091*	2.605 ± 0.025[a]	2.711 ± 0.071[ab]	2.833 ± 0.071[b]	0.016	0.915
FGR	2.054 ± 0.025*	2.228 ± 0.020[a]	2.118 ± 0.018[b]	2.082 ± 0.014[b]	< 0.001	0.102

*There were significant difference ($P < 0.05$) between the control and A groups (n = 60). The t test was performed to compare these 2 groups Value = Means ± SEM.

[a,b,c]Without same letters in the same line differ significantly ($P < 0.05$) in A, B, and C groups (n = 60). A one-way analysis of variation and Tukey's test were used to determine the differences among the 3 heat-stressed groups. Value = Means ± SEM.

[1]Control group: Chicken in control group were kept in the normal temperature environment and fed a basal diet.

[2]A, B, and C groups: Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 0, 100, and 200 mg/kg phloretin.

[3]The linear and quadratic effects of phloretin were detected by orthogonal polynomials. Abbreviations: IBW, initial body weight; FBW, final body weight; BWG, body weight gain; FI, feed intake; FGR, feed-to-gain ratio.

Table 4. Effect of phloretin on serum biochemical parameters in broilers (22−42 d) exposed to hot environment.

Item	Control[1]	Heat stress groups[2]			*P*-Value	
		A	B	C	Linear[3]	Quadratic[3]
TP (mg/mL)	31.67 ± 1.61*	26.21 ± 0.93^{a}	28.44 ± 0.99^{ab}	29.00 ± 0.72^{b}	0.010	0.727
Glucose (mmol/mL)	12.16 ± 0.33	11.75 ± 0.21	11.90 ± 0.24	11.99 ± 0.26	0.490	0.897
TG (mmol/L)	0.58 ± 0.02*	0.39 ± 0.02^{a}	0.47 ± 0.01^{ab}	0.51 ± 0.03^{b}	0.007	0.636
T3 (ng/mL)	1.44 ± 0.05*	1.07 ± 0.08	1.20 ± 0.05	1.26 ± 0.08	0.084	0.708
T4 (ng/mL)	10.70 ± 0.50*	8.20 ± 0.42^{a}	9.4 ± 0.31^{ab}	9.80 ± 0.32^{b}	0.006	0.359

*There were significant difference ($P < 0.05$) between the control and A groups (n = 12). The *t* test was performed to compare these 2 groups Value = Means ± SEM.

^{a,b,c}Without same letters in the same line differ significantly ($P < 0.05$) in A, B, and C groups (n = 12). A one-way analysis of variation and Tukey's test were used to determine the differences among the 3 heat-stressed groups. Value = Means ± SEM.

[1]Control group: Chicken in control group were kept in the normal temperature environment and fed a basal diet.

[2]A, B, and C groups: Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 0, 100, and 200 mg/kg phloretin.

[3]The linear and quadratic effects of phloretin were detected by orthogonal polynomials. Abbreviations: TP, total protein; TG, triglyceride; T3, triiodothyronine; T4, thyroxine.

Table 5. Effect of phloretin on serum redox state and antioxidants in broilers (22−42 d) exposed to hot environment.

Item	Control[1]	Heat stress groups[2]			*P*-Value	
		A	B	C	Linear[3]	Quadratic[3]
MDA (nmol/mL)	2.20 ± 0.14*	3.25 ± 0.25^{a}	2.83 ± 0.14^{ab}	2.47 ± 0.18^{b}	0.012	0.893
GSH (μmol/L)	25.17 ± 1.23*	20.04 ± 0.90^{a}	22.87 ± 1.03^{ab}	23.59 ± 0.54^{b}	0.010	0.327
SOD (U/mL)	176.63 ± 11.97	158.64 ± 4.76	170.79 ± 5.87	170.99 ± 6.46	0.149	0.409
CAT (U/mL)	3.55 ±0.11*	2.99 ± 0.12^{a}	3.23 ± 0.09^{ab}	3.40 ± 0.08^{a}	0.009	0.791
T-AOC (mM)	0.44 ± 0.01*	0.40 ± 0.01^{a}	0.43 ± 0.01^{b}	0.44 ± 0.01^{b}	0.005	0.279

*There were significant difference ($P < 0.05$) between the control and A groups (n = 12). The *t* test was performed to compare these 2 groups Value = Means ± SEM.

^{a,b,c}Without same letters in the same line differ significantly ($P < 0.05$) in A, B, and C groups (n = 12). A one-way analysis of variation and Tukey's test were used to determine the differences among the 3 heat-stressed groups. Value = Means ± SEM.

[1]Control group: Chicken in control group were kept in the normal temperature environment and fed a basal diet.

[2]A, B, and C groups: Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 0, 100, and 200 mg/kg phloretin.

[3]The linear and quadratic effects of phloretin were detected by orthogonal polynomials. Abbreviations: SEM, standard error of the mean; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; T-AOC, total antioxidant capacity.

Serum Oxidation State

Heat stress (A group) increased ($P < 0.05$) serum MDA level, but decreased ($P < 0.05$) serum GSH, CAT and T-AOC levels in broilers compared with that in the control group (Table 5). As shown in Table 5, broilers in the B group (heat stress + 100 mg/kg phloretin treatment) exhibited higher serum T-AOC than those in the A group (heat stress treatment); broilers in the C group (heat stress + 200 mg/kg phloretin treatment) exhibited lower serum MDA, but higher serum GSH, CAT and T-AOC than those in the A group (heat stress treatment). Under high temperature condition, MDA, GSH, CAT and T-AOC were linearly affected by inclusion of phloretin (Table 5).

GSH-Related Enzyme

Heat stress (A group) decreased ($P < 0.05$) the serum GSH-Px and γ-GCS levels in broilers compared with that in the control group (Table 6). As shown in Table 6, broilers in the C group (heat stress + 200 mg/kg

Table 6. Effect of phloretin on serum GSH-related enzymes in broilers (22−42 d) exposed to hot environment.

Item	Control[1]	Heat stress groups[2]			*P*-Value	
		A	B	C	Linear[3]	Quadratic[3]
GSH-Px (nm(U/mL)	667.08 ± 6.38*	625.63 ± 6.42^{a}	647.08 ± 6.36^{ab}	643.33 ± 1.87^{b}	0.033	0.073
γ-GCS (U/mL)	0.64 ± 0.05*	0.48 ± 0.03^{a}	0.53 ± 0.03^{ab}	061 ± 0.04^{b}	0.019	0.816

*There were significant difference ($P < 0.05$) between the control and A groups (n = 12). The *t* test was performed to compare these 2 groups Value = Means ± SEM.

^{a,b,c}Without same letters in the same line differ significantly ($P < 0.05$) in A, B, and C groups (n = 12). A one-way analysis of variation and Tukey's test were used to determine the differences among the 3 heat-stressed groups. Value = Means ± SEM.

[1]Control group: Chicken in control group were kept in the normal temperature environment and fed a basal diet.

[2]A, B, and C groups: Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 0, 100, and 200 mg/kg phloretin.

[3]The linear and quadratic effects of phloretin were detected by orthogonal polynomials. Abbreviations: GSH-Px, glutathione peroxidase; γ-GCS, γ-glutamylcysteine synthetase.

Table 7. Effect of phloretin on serum Nrf2 and HSP70 in broilers (22−42 d) exposed to hot environment.

Item	Control[1]	Heat stress groups[2]			*P*-Value	
		A	B	C	Linear[3]	Quadratic[3]
Nrf2 (nm(ng/mL)	0.63 ± 0.03*	0.50 ± 0.01[a]	0.56 ± 0.03[ab]	0.59 ± 0.02[b]	0.007	0.518
HSP70 (ng/mL)	4.06 ± 0.19*	4.89 ± 0.09[a]	4.35 ± 0.20[ab]	4.26 ± 0.17[b]	0.012	0.265

*There were significant difference ($P < 0.05$) between the control and A groups (n = 12). The *t* test was performed to compare these 2 groups Value = Means ± SEM.

[a,b,c]Without same letters in the same line differ significantly ($P < 0.05$) in A, B, and C groups (n = 12). A one-way analysis of variation and Tukey's test were used to determine the differences among the 3 heat-stressed groups. Value = Means ± SEM.

[1]Control group: Chicken in control group were kept in the normal temperature environment and fed a basal diet.

[2]A, B, and C groups: Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 0, 100, and 200 mg/kg phloretin.

[3]The linear and quadratic effects of phloretin were detected by orthogonal polynomials. Abbreviations: Nrf2, nuclear Factor Erythroid 2−Related 2; HSP70, Heat Shock Protein 70.

phloretin treatment) exhibited higher serum GSH-Px and γ-GCS than those in the A group (heat stress treatment). Under high temperature condition, serum GSH-Px and γ-GCS were linearly affected by inclusion of phloretin (Table 6).

Nrf2 and HSP70

Heat stress (A group) decreased ($P < 0.05$) the serum Nrf2 level, and increased HSP70 levels in broilers compared with that in the control group (Table 7). As shown in Table 7, broilers in the C group (heat stress + 200 mg/kg phloretin treatment) exhibited higher serum Nrf2, and lower HSP70 than those in the A group (heat stress treatment). Under high temperature condition, serum Nrf2 and HSP70 were linearly affected by inclusion of phloretin (Table 7).

DISCUSSION

High summer temperatures seriously affect broiler production (Lu et al., 2019; Humam et al., 2019). Feeding is an essential life activity to ensure survival and production of animals. Adequate FI is important for improving animal survival and production potential. In high temperature environments, the physiological functions of the body will change, and with the increase in stress intensity, metabolism will become disordered and FI will decrease, which will result in the loss of body weight (or even negative growth), reducing immune function and causing death. Liu et al. (2019) showed that a decreased FI led directly to the decline in body weight and feed utilization in heat-stressed broilers. Consistent with the results of previous studies, we showed that continuous heat stress significantly reduced the FBW, BWG, and FI, proving that continuous heat stress reduces the growth rate and feed utilization efficiency of broilers. Several studies have shown that adding antioxidants such as vitamin E, epigallocatechin gallate, and curcumin to the diet can alleviate heat stress and maintain broiler performance (Zhang et al., 2015a; Mazur-Kuśnirek et al., 2019; Xue et al., 2017). Similarly, phloretin can reverse the negative effects of growth performance in broilers exposed to hot environments in this study. Zhang et al. (2015b) showed that the plant antioxidant, curcumin, can also improve the growth performance of heat-stressed broilers.

Blood indicators are widely used to study the effects of heat stress on the body (Chand et al., 2018). The commonly used serum glucose, TG, and TP contents reflect sugar, protein, and fat metabolism, and serum T3 and T4 contents reflect the expression of thyroxine in animals (Willemsen et al., 2011; Luo et al., 2018; Ghasemi and Nari, 2020). Generally, heat stress leading to a decrease in broiler FI, and insufficient nutritional intake will inevitably cause the body to accelerate the catabolism of reserves to ensure sufficient energy supply, thus, reducing the blood protein and lipid levels (Luo et al., 2018). In addition, long-term high temperature environment will also interfere with the animal's endocrine system, causing disorders of serum hormone levels. T3 and T4 are important hormones that regulate the metabolic rate and are sensitive indicators of the state of the stress response (Willemsen et al., 2011). The results of the present study showed that serum T3 and T4 hormones were decreased in broilers during heat stress. However, dietary supplementation of phloretin can increase the levels of serum TP, TG, T3, and T4 in heat-stressed broilers. Adding phloretin to the diet can increase the utilization of TP and TG, effectively inhibiting the catabolism of reserve TP and TG, thereby alleviating the effect of high temperature stress on serum biochemical indicators in broilers.

During oxidative stress, cellular reactive oxygen species is generated and the by-products of oxidation reactions are accumulated. The endogenous antioxidant defense system also affects the redox state of the tissue. The elimination of free radicals in the body depends on various antioxidant factors in the body, including GSH, SOD, and CAT (Bai et al., 2019; Zhang et al., 2020). High temperature environments will significantly increase the degree of serum peroxidation in broilers, accelerate the depletion of serum antioxidant factors, and destroy the body's antioxidant capacity (Bai et al., 2019; Wan et al., 2017). Consistent with these results, the present study showed that heat stress significantly reduced the expression of serum antioxidant factors in broilers, while phloretin can increase the concentration of these factors. The ability of phloretin to relieve the

body's oxidative stress is related to its effect on inducing changes in the redox state of cells (Nithiya and Udayakumar 2016; Han et al., 2020). Huang et al. (2017) showed that phloretin could significantly increase the concentration of GSH, and reduce the level of MDA in oxidative-stressed lungs of asthmatic mice. Zhang et al. (2019) also suggested that phloretin have improvement of oxidative stress in the colon of mice with ulcerative colitis through the regulation of MDA, SOD, and GSH levels. Thus, the possible reason how phloretin can alleviate heat stress damage is that it enhances the body's antioxidant effect.

GSH-related enzymes also participate in the regulation of natural plant antioxidants during cell oxidative stress (Zhang et al. 2018a). Under normal physiological conditions, the synthesis and decomposition of GSH in cells are in equilibrium. The enzyme, γ-GCS, is the key rate-limiting enzyme for GSH synthesis, which catalyzes the acceleration of GSH synthesis and increases the cell GSH concentration (Zhang et al. 2018a). GSH-Px (peroxidase decomposing enzyme) is an important antioxidant enzyme in the cell (Zhang et al. 2018a). It is mainly responsible for catalyzing reduced GSH to oxidized glutathione in the turnover metabolism of GSH; in this process (toxic peroxidation) the substance is finally transformed into a nontoxic or more stable hydroxy compound. In the present study, we showed that phloretin can indeed regulate the level of GSH by intervening in the metabolic pathway of GSH. Based on this, we speculated that the antioxidant activity of phloretin is closely related to the regulation of GSH level, and this regulation is likely to be achieved by coordinating the synthesis and decomposition pathways of GSH. Huang et al. (2017) and Zhang et al. (2019) both showed that the GSH was raised in the oxidative -stressed lung and colon of mice by phloretin addition.

Nrf2 factor plays an important role in the antioxidant effect of tissue cells (Zhang et al., 2015b; Hu et al., 2020a). During oxidative stress, Nrf2 binds to antioxidant response element, activates the promoter, and increases the expression of downstream related antioxidant enzyme genes (Hu et al., 2020a). Natural plant antioxidants such as curcumin, resveratrol, and epigallocatechin gallate can increase the expression of antioxidant enzyme genes by activating the Nrf2 protein (Zhang et al., 2015b; Xue et al., 2017; Zhang et al., 2018b). Ying et al. (2018) revealed that phloretin attenuated the serum oxidative stress damage and pathological parameters via Nrf2 pathway in diabetic mice. Liu et al. (2015) reported that phloretin improved neuronal oxidative stress via activation of the Nrf2 defense pathway in cerebral ischemia/reperfusion rats. In the current study, phloretin also increased the expression level of serum antioxidant factors in heat-stressed broilers may through the Nrf2 activation. In addition, HSP70 is an important response protein to heat stress and oxygen stress (Hu et al., 2020b). HSP70 is a heat shock protein that is widely distributed in various cells and tissues. When the body encounters an unfavorable environment or physiological stimuli, the expression of HSP70 will increase significantly (Gu et al., 2012; Zhang et al., 2015a). In the present study, the expression of HSP70 increased significantly during the continuous high temperatures of summer, and the dietary supplementation of phloretin significantly inhibited the production of HSP70, indicating that the antioxidant properties of phloretin alleviate heat stress damage in broilers. This is consistent with the results of other studies that plant antioxidants alleviate the negative effects (such as low growth performance) of heat stress in animal (Zhang et al., 2015a; Liu et al., 2016).

In conclusion, heat stress significantly impaired growth performance, serum biochemical parameters and antioxidant system of broilers. Dietary supplementation of phloretin could increase the level of antioxidant factors by activating Nrf2 protein expression and GSH-related enzyme activities, which reduce oxidative damage in heat-stressed broilers. Our results suggested that phloretin, as a natural plant antioxidant, may ameliorate the heat-stress-impaired production performance and serum indicators through the improved redox status in broilers.

ACKNOWLEDGMENTS

This research was funded by Open Fund of State Key Laboratory of Animal Nutrition (No. 2004DA125184F1910), the Natural Science Foundation of Anhui Provincial Department of Education (No. KJ2020A0084), National Natural Science Foundation of China (No. 32002223 and 31601983), Agricultural Science and Technology Innovation Program (CAAS-ZDRW202006-02, ASTIP-IAS07), and Initial Scientific Research Foundation for Talents of Anhui Science and Technology University (DKYJ201802).

DISCLOSURES

The authors have declared that no competing interests exist.

REFERENCES

Akbarian, A., J. Michiels, J. Degroote, M. Majdeddin, A. Golian, and S. De Smet. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. J. Anim. Sci. Biotechno. 7:37.

Barreca, D., E. Bellocco, G. Lagana, G. Ginestra, and C. Bisignano. 2014. Biochemical and antimicrobial activity of phloretin and its glycosilated derivatives present in apple and kumquat. Food Chem. 160:292–297.

Behzad, S., A. Sureda, D. Barreca, S. F. Nabavi, L. Rastrelli, and S. M. Nabavi. 2017. Health effects of phloretin: from chemistry to medicine. Phytochem. Rev. 16:527–533.

Bai, X., S. Dai, J. Li, S. Xiao, A. Wen, and H. Hu. 2019. Glutamine improves the growth performance, serum biochemical profile and antioxidant status in broilers under medium-term chronic heat stress. J. Appl. Poult. Res. 28:1248–1254.

Chang, W., W. Huang, and C. Liou. 2012. Evaluation of the anti-inflammatory effects of phloretin and phlorizin in lipopolysaccharide-stimulated mouse macrophages. Food Chem. 134:(2), 972–979.

Chand, N., S. Naz, Z. Rehman, and R. U. Khan. 2018. Blood biochemical profile of four fast-growing broiler strains under high ambient temperature. Appl. Biol. Chem. 61:273–279.

Farag, M. R., and M. Alagawany. 2018. Physiological alterations of poultry to the high environmental temperature. J. Therm. Biol. 76:101–106.

Gu, X., Y. Hao, and X. Wang. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poult. Sci. 91:790–799.

Ghazi, S., T. Amjadian, and S. Norouzi. 2015. Single and combined effects of vitamin C and oregano essential oil in diet, on growth performance, and blood parameters of broiler chicks reared under heat stress condition. Int. J. Biometeorol. 59:1019–1024.

Goo, D., J. H. Kim, G. H. Park, J. B. Delos Reyes, and D. Y. Kil. 2019. Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals 9:107.

Ghanima, M. M. A., M. E. A. El-Hack, S. I. Othman, A. E. Taha, A. A. Allam, and A. M. E. Abdel-Moneim. 2020. Impact of different rearing systems on growth, carcass traits, oxidative stress biomarkers, and humoral immunity of broilers exposed to heat stress. Poult. Sci. 99:3070–3078.

Ghasemi, H. A., and N. Nari. 2020. Effect of supplementary betaine on growth performance, blood biochemical profile, and immune response in heat-stressed broilers fed different dietary protein levels. J. Appl. Poult. Res. 29:301–313.

Han, L., J. Li, J. Li, C. Pan, Y. Xiao, X. Lan, and M. Wang. 2020. Activation of AMPK/Sirt3 pathway by phloretin reduces mitochondrial ROS in vascular endothelium by increasing the activity of MnSOD via deacetylation. Food Funct. 11:3073–3083.

Huang, W., L. Fang, and C. Liou. 2017. Phloretin attenuates allergic airway inflammation and oxidative stress in asthmatic mice. Front. Immunol. 8:134.

Humam, A. M., T. C. Loh, H. L. Foo, A. A. Samsudin, N. M. Mustapha, I. Zulkifli, and W. I. Izuddin. 2019. Effects of feeding different postbiotics produced by Lactobacillus plantarum on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. Animals 9:644.

Hu, H., S. Dai, A. Wen, and X. Bai. 2019. Efficient expression of xylanase by codon optimization and its effects on the growth performance and carcass characteristics of broiler. Animals 9:65.

Hu, H., S. Dai, J. Li, A. Wen, and X. Bai. 2020a. Glutamine improves heat stress-induced oxidative damage in the broiler thigh muscle by activating the nuclear factor erythroid 2-related 2/Kelch-like ECH-associated protein 1 signaling pathway. Poult. Sci. 99:1454–1461.

Hu, H., L. Chen, S. Dai, J. Li, and X. Bai. 2020b. Effect of glutamine on antioxidant capacity and lipid peroxidation in the breast muscle of heat-stressed broilers via antioxidant genes and HSP70 pathway. Animals 10:404.

Li, X., Q. Wang, L. Liang, X. Chen, K. Bi, R. Dai, and Y. Liu. 2013. Determination of phloretin in rat plasma by HPLC and application to pharmaco-kinetic study. Chin. Tradit. Pat. Med. 3:487–490.

Lin, C., C. Chu, C. Ng, C. Lin, D. Chen, I. Pan, and K. Huang. 2014. Immunomodulation of phloretin by impairing dendritic cell activation and function. Food Funct. 5:997–1006.

Liu, Y., L. Zhang, and J. Liang. 2015. Activation of the Nrf2 defense pathway contributes to neuroprotective effects of phloretin on oxidative stress injury after cerebral ischemia/reperfusion in rats. J. Neurol. Sci. 351:88–92.

Liu, L., C. Fu, M. Yan, H. Xie, S. Li, Q. Yu, S. He, and J. He. 2016. Resveratrol modulates intestinal morphology and HSP70/90, NF-κB and EGF expression in the jejunal mucosa of black-boned chickens on exposure to circular heat stress. Food Funct. 7:1329–1338.

Liu, W., Y. Yuan, C. Sun, B. Balasubramanian, Z. Zhao, and L. An. 2019. Effects of dietary betaine on growth performance, digestive function, carcass traits, and meat quality in indigenous yellow-feathered broilers under long-term heat stress. Animals 9:506.

Luo, J., J. Song, L. Liu, B. Xue, G. Tian, and Y. Yang. 2018. Effect of epigallocatechin gallate on growth performance and serum biochemical metabolites in heat-stressed broilers. Poult. Sci. 97:599–606.

Lu, Z., X. He, B. Ma, L. Zhang, J. Li, Y. Jiang, G. Zhou, and F. Gao. 2019. The alleviative effects and related mechanisms of taurine supplementation on growth performance and carcass characteristics in broilers exposed to chronic heat stress. Poult. Sci. 98:878–886.

Mendes, R. A., B. L. Silva, R. Takeara, R. G. Freitas, A. Brown, and G. L. De Souza. 2018. Probing the antioxidant potential of phloretin and phlorizin through a computational investigation. J. Mol. Model. 24:101.

Mazur-Kuśnirek, M., Z. Antoszkiewicz, L. Krzysztof, J. Kaliniewicz, S. Kotlarczyk, and P. Żukowski. 2019. The effect of polyphenols and vitamin E on the antioxidant status and meat quality of broiler chickens exposed to high temperature. Arch Anim Nutr. 73:111–126.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. National Academic Press, Washington, DC.

Nithiya, T., and R. Udayakumar. 2016. In Vitro antioxidant properties of phloretin-An important phytocompound. J. Biosci. Med. 4:85–94.

Nawab, A., F. Ibtisham, G. Li, B. Kieser, J. Wu, W. Liu, Y. Zhao, Y. Nawab, K. Li, M. Xiao, and L. An. 2018. Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. J. Therm. Biol. 78:131–139.

Shao, X., N. Bai, K. He, C. Ho, C. S. Yang, and S. Sang. 2008. Apple polyphenols, phloretin and phloridzin: new trapping agents of reactive dicarbonyl species. Chem. Res. Toxicol. 21:2042–2050.

Shakeri, M., J. J. Cottrell, S. Wilkinson, H. H. Le, H. A. R. Suleria, R. D. Warner, and F. R. Dunshea. 2019. Growth performance and characterization of meat quality of broiler chickens supplemented with betaine and antioxidants under cyclic heat stress. Antioxidants 8:336.

Slawinska, A., M. Zampiga, F. Sirri, A. Meluzzi, M. Bertocchi, S. Tavaniello, and G. Maiorano. 2020. Impact of galactooligosaccharides delivered in ovo on mitigating negative effects of heat stress on performance and welfare of broilers. Poult. Sci. 99:407–415.

Wan, X., L. Jiang, H. Zhong, Y. Lu, L. Zhang, and T. Wang. 2017. Effects of enzymatically treated *Artemisia annua* L. on growth performance and some blood parameters of broilers exposed to heat stress. Anim. Sci. J. 88:1239–1246.

Wang, H., T. Yang, T. Wang, N. Hao, Y. Shen, Y. Wu, Z. Yuan, L. Chen, and F. Wen. 2018. Phloretin attenuates mucus hypersecretion and airway inflammation induced by cigarette smoke. Int. Immunopharmacol. 55:112–119.

Wang, F., X. Xiao, Y. Yuan, J. Liu, Y. Liu, and X. Yi. 2020. Solubilization of phloretin via steviol glycoside-based solid dispersion and micelles. Food Chem 308 125569.

Willemsen, H., Q. Swennen, N. Everaert, P. Geraert, Y. Mercier, A. Stinckens, E. Decuypere, and J. Buyse. 2011. Effects of dietary supplementation of methionine and its hydroxy analog DL-2-hydroxy-4-methylthiobutanoic acid on growth performance, plasma hormone levels, and the redox status of broiler chickens exposed to high temperatures. Poult. Sci. 90:2311–2320.

Xue, B., J. Song, L. Liu, J. Luo, G. Tian, and Y. Yang. 2017. Effect of epigallocatechin gallate on growth performance and antioxidant capacity in heat-stressed broilers. Arch. Anim. Nutr. 71:362–372.

Ying, Y., J. Jin, L. Ye, P. Sun, H. Wang, and X. Wang. 2018. Phloretin prevents diabetic cardiomyopathy by dissociating Keap1/Nrf2 complex and inhibiting oxidative stress. Front. Endocrino. 9:774.

Zuo, A., Y. Yanying, J. Li, X. Binbin, Y. Xiongying, Q. Yan, and C. Shuwen. 2011. Study on the relation of structure and antioxidant activity of isorhamnetin, quercetin, phloretin, silybin and phloretin isonicotinyl hydrazone. Free Radicals Antioxid. 1:39–47.

Zhang, J., Z. Hu, C. Lu, K. Bai, L. Zhang, and T. Wang. 2015a. Effect of various levels of dietary curcumin on meat quality and antioxidant profile of breast muscle in broilers. J. Agr. Food Chem. 63:3880–3886.

Zhang, J., Z. Hu, C. Lu, K. M. Yang, L. Zhang, and T. Wang. 2015b. Dietary curcumin supplementation protects against heat-stress-impaired growth performance of broilers possibly through a mitochondrial pathway. J. Anim. Sci. 93:1656–1665.

Zhang, J., K. Bai, W. Su, A. Wang, L. Zhang, K. Huang, and T. Wang. 2018a. Curcumin attenuates heat-stress-induced oxidant damage by simultaneous activation of GSH-related antioxidant enzymes and Nrf2-mediated phase II detoxifying enzyme systems in broiler chickens. Poult. Sci. 97:1209–1219.

Zhang, C., K. Chen, X. Zhao, and Z. Geng. 2018b. Protective effects of resveratrol against high ambient temperature-induced spleen dysplasia in broilers through modulating splenic redox status and apoptosis. J. Sci. Food. Agr. 98:5409–5417.

Zhang, C., C. Wang, X. Zhao, K. Chen, and Z. Geng. 2020. Effect of L-theanine on meat quality, muscle amino acid profiles, and antioxidant status of broilers. Anim. Sci. J. 91:e13351.

Zhang, Z., S. Li, H. Cao, P. Shen, J. Liu, Y. Fu, Y. Cao, and N. Zhang. 2019. The protective role of phloretin against dextran sulfate sodium-induced ulcerative colitis in mice. Food Funct 10:422–431.